J. Cosmet. Sci., 67, 185–203 (May/June 2016)

Effects of season on stratum corneum barrier function and skin biomarkers

KARL SHIQING WEI, CHING STELLA,
KENNETH R. WEHMEYER, JEREMY CHRISTMAN,
AMY ALTEMEIER, RUSSELL SPRUELL,
ROHAN L. WIMALASENA, GINA M. FADAYEL,
RAYMOND A. REILMAN, SAFA MOTLAGH,
PETER J. STOFFOLANO, KATHLEEN BENZING, and
R. RANDALL WICKETT, *The Procter & Gamble Company, Cincinnati, OH (K.S.W., C.S., K.R.W., J.C., A.A., R.S.L., R.L.W., G.M.F., R.A.R., S.M., P.J.S., K.B.) and James L. Winkle College of Pharmacy, Cincinnati, OH (R.R.W.)*

Accepted for publication July 12, 2016.

Synopsis

The skin on the lower legs of 25 female subjects was evaluated first in the winter, and then again in the summer of the same subjects. Barrier function was determined by measuring transepidermal water loss (TEWL), and skin hydration and dryness were evaluated by electrical measurements (Corneometer® CM825) and visual grading. Stratum corneum (SC) was sampled using 10 sequential D-Squame sampling discs and analyzed for 2-pyrrolidone-5-carboxylic acid (PCA), keratin-1,10,11, interleukin 1α (IL-1α), interleukin 1 receptor antagonist (IL-1ra), selected ceramides, cholesterol, cholesterol sulfate, and selected free fatty acids. TEWL as well as the visual dryness grades were significantly lower in the summer while hydration was higher. PCA was significantly higher in the summer as were the keratins. The ratio IL-1ra:IL-1α, an indicator of skin inflammation, was significantly lower in the summer. The amount of protein removed by the tape strips was also significantly lower in summer indicating better SC cohesion. Among the SC lipids measured, total ceramides, individual ceramides, total fatty acids, and cholesterol were higher in summer compared to winter. Stearic acid and cholesterol sulfate were not significantly different between winter and summer.

INTRODUCTION

It is well known that dry skin develops more readily in the winter than in the summer. In addition, skin is more responsive to irritants, such as surfactants in cleansing products, during winter as shown in studies involving exaggerated use of washing products (1–3) and on workers who repeatedly use cleansers in their occupations (4–6).

Address all correspondence to Randy Wickett at wickterr@ucmail.uc.edu.

Previous studies have investigated seasonal differences in biophysical properties of the skin in different populations. Hydration measured by electrical methods has been found to be consistently higher in summer (7–13) but transepidermal water loss (TEWL) differences have not been as consistent. Black *et al.* (8) reported higher TEWL in summer on the forearm, calf and crows-foot areas whereas Kikuchi *et al.* (10) reported lower TEWL in summer on the forearms and cheeks of both pre- and postmenopausal Japanese women. Seasonal differences were more marked on the face. Wan *et al.* reported higher TEWL on the foreheads of Chinese women in autumn and winter compared to spring and summer (7). Muizzuddin *et al.* reported lower TEWL on the cheek in summer versus winter in subjects living in arid (Arizona) and humid (New York) climates (11).

Egawa and Tagami used *in vivo* confocal Raman spectroscopy to investigate seasonal differences in stratum corneum (SC) components with Japanese subjects (9). Natural moisturizing factor (NMF) components, defined as free amino acids and 2-pyrrolidone-5-carboxylic acid (PCA), were not higher in summer compared to other seasons. Urea and lactate were found to be higher in summer, and trans-urocanic acid (t-UA) was found to be lower in summer. Using a tape stripping method on the forearms of Japanese males, Nakagawa *et al.* also found higher values of urea and lactate in the SC in the summer than in the winter (14).

Seasonal changes in SC structural lipids that may contribute to the difference in summer and winter skin condition have been reported in the literature. Increased levels of ceramides and cholesterol have been reported in summer (9,15,16). Ishikawa *et al.* reported changes in the ceramide profiles in summer compared to winter, and further that capacitance was directly proportional and TEWL inversely proportional to total ceramide level (17). Capacitance was higher in summer on all body sites, but TEWL differences varied with body site.

Recently, cytokines such as interleukin 1-α (IL-1α) and interleukin 1 receptor antagonist (IL-1ra) and structural proteins (involucrin and keratin-1,10,11) have been studied as biomarkers of skin health (18–22). The ratio of IL-1ra:IL-1α has been found to increase in skin treated with sodium lauryl sulfate (18,19), and the structural proteins keratin-1,10,11 were reported to be lower in the irritated skin of health-care workers (22). Involucrin was found to be lower and keratin-1,10,11 to be higher in adults' skin compared with infants' skin (21). Effective treatment decreased the IL-1ra:IL-α ratio and increased keratin-1,10,11 on the scalp of dandruff sufferers (20). To our knowledge, seasonal differences in these biomarkers have not yet been reported in the literature.

The goal of the present study was to evaluate the effect of season on skin health by skin grading, biophysical measurement, and noninvasive sampling for NMF components, biomarkers, and SC lipids. Female subjects were given a mild synthetic detergent bar to use for a one-week prewash during the winter. Subjects who developed at least moderately dry skin on the lower legs were selected for evaluation. The same subjects returned for evaluation during the following summer. After the same prewash procedure, the subjects underwent the same evaluations as in the winter study. Biophysical properties measured were TEWL and hydration (Corneometer). Visual grading was performed to evaluate skin dryness and redness. SC was sampled using 10 sequential D-Squame sampling discs (CUDERM, Dallas, TX) to analyze for SC cohesiveness, NMF components, biomarkers (IL-1ra:IL-α, involucrin, keratin-1,10,11), and SC lipids. The study protocol allowed for direct paired comparison of the same subjects in summer and winter for all properties measured.

MATERIALS AND METHODS

SUBJECTS

Healthy female subjects between 18 and 65 years of age with Fitzpatrick skin types II–IV (23) were given a mild synthetic cleansing bar to use at home daily on the lower legs for a 7-day prewash during the winter season in Cincinnati, OH. The study was approved by an institutional review board, and informed consent was obtained from all subjects.

At the end of the prewash, skin dryness and erythema on the legs were graded by an expert grader using previously published 0–6 scales (24). Subjects with a dry skin grade of at least 2.0 were selected for the study. Demographics of the 25 subjects selected for the study are presented in Table I below.

BIOPHYSICAL MEASUREMENTS AND SC SAMPLING

Measurements of skin hydration were obtained using a Corneometer® CM 825 (Courage + Khazaka, Cologne, Germany), and TEWL was measured using a Dermalab® Evaporimeter (Cortex Technologies, Hadslund, Denmark). Biophysical measurements were made after at least 30 min of equilibration in a controlled environment room with temperature (70° ± 2°F) and relative humidity 30–45%. SC from the outer aspect of the lower legs was sampled using 10 successive D-Squame Standard Sampling Discs (D100; CuDerm Corporation, Dallas, TX). Each sampling disc was pressed down onto the site using the D-Squame Pressure Instrument (D500; CuDerm Corporation) for 5 s, then removed from the skin and placed into 12-well collection plates. The discs were analyzed for total protein, PCA, IL-1α, IL-1ra, keratin-1,10,11, and lipids, including selected ceramides, selected fatty acids, cholesterol, and cholesterol sulfate. Two sites on each leg were sampled, and data were averaged at each tape strip for each subject.

The same 25 panelists returned in the summer, and same female subjects, after going through a 7-day prewash, were assessed by visual grading, biophysical measurements, and biomarker analysis exactly as mentioned above. The average outdoor temperature during the winter study was 4.4°C. The average temperature during the summer study was 21.8°C.

Table I
Subject Demographics

Number	25
Age (years)	47.6 ± 10
Age range	23–64
Fitzpatrick skin type	Number
II	3
III	18
IV	4

D-SQUAME ANALYSIS SCHEME

The protein content of all D-Squame sampling discs was analyzed nondestructively by measuring the optical absorption with a SquameScan™ 850A infrared densitometer (Heiland Electronic, Wetzlar, Germany). The device measures optical absorption at 850 nm, which is linearly related to protein content of the D-Squame sample (25). SC cytokines (IL-1α and IL-1ra) were measured using tape 2, and NMF components were measured using tape 3 and tape 10. Structural proteins (involucrin, keratin-1,10,11) were measured on tape 4, and SC lipids were measured using tapes 6 and 7 pooled together for better sensitivity. Measurements for cytokines, NMF, and structural proteins were normalized to protein measured by the Pierce® BCA protein assay (Thermo Scientific, Rockford, IL) and lipids were normalized to SquameScan™ values.

Analysis of NMFs from D-Squame discs. NMF samples (L-citrulline, glycine, L-ornithine, L-proline, 2-pyrrolidone-5-carboxylic acid, L-serine, t-UA, and L-histidine) collected on D-Squame discs were prepared for analysis by placing the discs into 2-ml polypropylene tubes with the glue side facing inward. A 25-µl aliquot of an internal standard solution (L-citrulline-D_7; glycine-D2,^{15}N; histidine-D_3; L-ornithine-D_6; L-proline-D3; 2-pyrrolidone-5-carboxylic-D_5 acid; L-serine-D3; cis-urocanic-$^{13}C_3$ acid) was added to each tube followed by 1.0 ml of water containing 0.1% formic acid and 0.1% heptafluorobutyric acid. The tubes were capped, vortexed for 10 s and then placed on a sonicator for 10 min. An aliquot of the extraction solution was removed for analysis by gradient reverse-phase high-performance liquid chromatographic analysis on a Waters Atlantis T3 (Milford, MA) column (2.1 x 50 mm, 3-µm particles). Detection and quantitation was performed using tandem mass spectrometry (MS/MS; Sciex AB-5000) operating under multiple reaction monitoring conditions for each analyte and the corresponding internal standard. Calibration standards prepared in 1.0 ml of water containing 0.1% formic acid and 0.1% heptafluorobutyric acid were used to generate regression curves for each NMF by plotting the peak area ratio for a given NMF standard (peak area NMF/peak area for internal standard) versus the standard concentration. The concentration of a given NMF in the study samples was determined from its corresponding peak area ratio by interpolation from the regression curve. The nominal range of quantitation is 20–20,000 ng/ml (20–20,000 ng/tape strip) for each NMF. The concentration of each NMF determined in the acid extract was converted into mass NMF/strip by multiplying by the extraction volume. The calculated mass of each NMF was then normalized by the protein amount in the acid extract determined by BCA assay using bovine serum albumin (BSA) as a standard.

Analysis of IL-1α and IL-1ra from D-Squame discs. Human inflammatory cytokines were analyzed to evaluate skin irritation and inflammatory processes. D-Squame discs collected from subjects were extracted with phosphate-buffered saline (PBS) containing an additional 0.25 M NaCl and a commercially available protease inhibitor cocktail containing a mixture of protease inhibitors with broad-spectrum inhibitory specificity (Roche Applied Science, Inc., Indianapolis, IN) for 30 min with sonication on ice. The extracts were then centrifuged for 5 min at 2100 × g to remove skin solids that might interfere in the assay. Aliquots of these extracts were then analyzed for soluble protein using the BCA Protein Assay Kit and BSA as a reference standard. After protein analysis, extracts were supplemented with 2% BSA, transferred into 96-well polypropylene deep-well plates and frozen at -80°C for cytokine analysis. Multiple human cytokines (IL-1α and IL-1ra) were simultaneously quantitated using a Milliplex Human Cytokine Multiplex Immunoassay Kit (Millipore Corp., Billerica, MA).

Analysis of skin proteins from D-Squame discs. D-Squame discs were extracted with PBS containing 0.2% sodium dodecyl sulfate and 0.5% propylene glycol for 30 min with sonication on ice. The extracts were then centrifuged for 5 min at 2100 × g to remove skin solids that might interfere in the assay. Subsequently, the extracts of D-Squame discs were transferred into 96-well polypropylene deep-well plates and frozen at -80°C for skin multiple analyte profile (SkinMAP) and soluble protein analyses. Human skin proteins (keratin-1,10, involucrin, human serum albumin) were simultaneously quantified using a 3-plex Human Skin Panel Multiplex Immunoassay Kit (Millipore Corp.). The antibody for human involucrin recognizes non-cross-linked involucrin protein, but may have reactivity with involucrin within the cornified envelope. Soluble protein was measured using BCA Protein Assay Kit.

Analysis of skin lipids from D-Squame discs. An array of skin lipids (cholesterol, cholesterol sulfate, selected fatty acids, and selected ceramides—see Table III for ceramide nomenclature) was determined from extracts of D-Squame discs containing samples from human skin using gradient supercritical fluid chromatography (SFC) with MS/MS with detection in the positive and negative ionization modes depending on the analyte using atmospheric pressure chemical ionization (APCI). The tape strips were first analyzed via a SquameScan™ 850A infrared densitometer to determine the amount of removed skin for normalization of the measured lipids. Two tape strips from each subject were transferred to 20-ml glass vials, spiked with an internal standard mixture [D_6-cholesterol, D_7-cholesterol sulfate, D_{47}-tetradecanoic acid, D_3-heptadecanoic acid, D_7-sphinanine, and D_{31}-*N*-palmitoyl-1-D-erythro-sphingosine (D_{31}-Ceramide)] and extracted using 3 ml of methanol followed by sonication at ambient temperature. The vials were centrifuged, and the methanol layer removed and placed in separate glass vial. The tape strips were then extracted with 3 ml of hexane followed by sonication for 15 min at ambient temperature, and the hexane layer was separated. The hexane and methanol layers for each set of tapes were then combined, dried under nitrogen at 50°C, and finally reconstituted in chloroform:MeOH (3:1; v/v). Standards [myristic acid, palmitic acid, palmitoleic acid, octadecanoic acid, oleic acid, linoleic acid, docosanoic acid, tetracosanoic acid, cholesterol, cholesterol sulfate, N(24_0)P(18), N(24_0)DS(18), A(16_0)S(18), A(24_0)P(18), ceramide EOS-C30, S(18)] were prepared in chloroform:MeOH (3:1) over a range of appropriate concentrations. The standards, spiked with internal standard, and the reconstituted samples were analyzed by gradient SFC with MS/MS detection using APCI. The fatty acids were monitored in the negative ion mode while selected ceramides, sphingoid bases, cholesterol, and cholesterol sulfate were monitored in the positive ion mode. The peak area ratio (standard peak area/internal standard peak area) for each standard level were used to construct a linear regression curve for each of the standard analytes. For analytes, where the standard was available (fatty acids, cholesterol, cholesterol sulfate, sphingoid bases), the actual standard was used, whereas for the ceramides the surrogate ceramide for the particular class was used. The lipid mass found for each analyte was divided by the SquameScan values for the corresponding tapes.

STATISTICAL METHODS: SKINMAP, NMF, CYTOKINES, AND LIPIDS

For each of the four sites within a subject, the SkinMAP (tape 4), NMF (tapes 3 and 10), cytokines (tape 2), and lipids (tape 6 and 7 pooled) measures were normalized to their associated protein measures (BCA for SkinMAP, NMF, and cytokines and SquameScan™

for lipids) for each study and log to the base 10 was taken. For values below quantifiable limits (BQLs), half the detection limit was substituted. Following normalization and log transformation, the SkinMAP, NMF, and cytokine data were averaged across all four sites for each subject. Thus, each subject has a summer and a winter measure. These data were then modeled with a mixed model analysis of covariance (ANCOVA) with fixed effect for time of year (group) and random effect for subject. All statistical tests comparing the two groups (winter vs. summer) were two sided using $\alpha = 0.05$ significance level.

The least squares (adjusted for model) mean (on the log scale), the original mean (before log transformation), *p* value testing for significant difference in each group mean from zero, standard errors, and *p* values for testing between group differences were calculated and reported. BQLs are reported and flagged for greater than 30%.

STATISTICAL METHODS: BIOPHYSICAL MEASURES AND VISUAL GRADES

The Corneometer, TEWL, Expert Dryness, and Expert Redness data were each averaged across all four sites within the marked skin area for each subject. Thus, each subject has a summer and a winter measure. These data were then modeled with a mixed model ANCOVA with fixed effect for time of year (group) and random effect for subject. All statistical tests comparing the two groups (winter vs. summer) were two sided using $\alpha = 0.05$ significance level.

RESULTS AND DISCUSSION

BIOPHYSICAL MEASURES

Electrical measurements for the evaluation of SC hydration are well established, and several instruments are available (26–28) including the Corneometer (29) which has been shown to give reading correlated to dry skin grades (30). The most common way to assess skin barrier function is through the measurement of TEWL (31–35).

Corneometer and TEWL results in summer and winter are shown in Figure 1A and B.

Corneometer readings were higher in summer (Figure 1A), indicating a higher level of skin hydration in agreement with the literature (7–13). TEWL was lower in summer compared to winter in agreement with the results reported by Muizzuddin *et al.* (11), Kikuchi *et al.* (10), and Wan *et al.* (7), but in contrast with those reported by Black *et al.* (8) who found higher TEWL values on the legs and forearms in July compared to February and December. Ishikawa *et al.* reported lower TEWL on the cheeks in summer compared to winter and lower TEWL on the buttocks in spring. On the palm, TEWL values were higher in autumn than in spring, but there were no difference on other body sites investigated including legs (17). In the present study, subjects with at least moderately dry skin on the legs after the wash-out period in winter were selected. This may have increased the difference in barrier function seen between winter and summer and led to the highly significant reduction in TEWL in summer seen in Figure 1B.

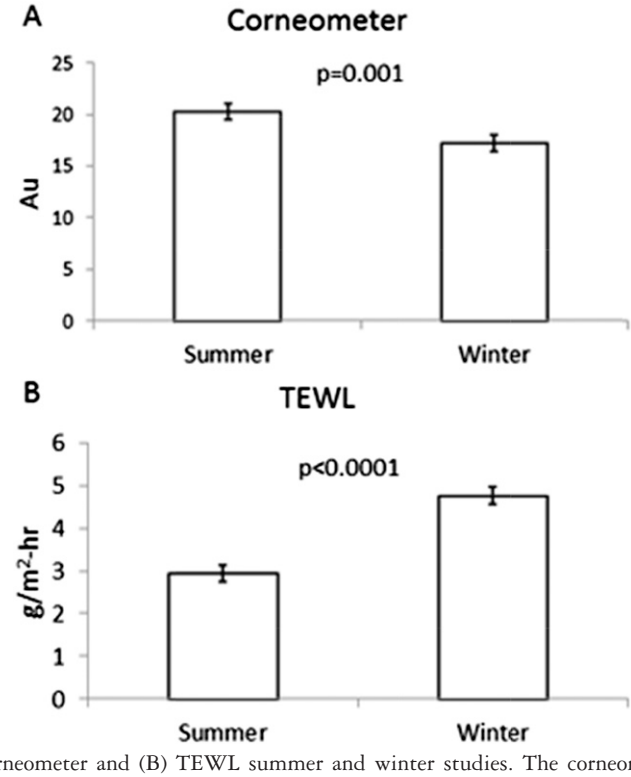

Figure 1. (A) Corneometer and (B) TEWL summer and winter studies. The corneometer readings were significantly higher and TEWL significantly lower in summer compared to winter.

VISUAL GRADES

Visual grades for dryness and erythema are shown in Figure 2. Dryness grades were significantly higher in winter, but erythema grades were not significantly different between the two seasons.

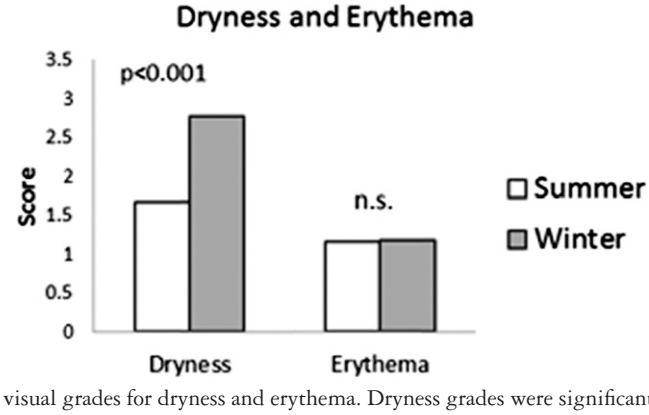

Figure 2. Expert visual grades for dryness and erythema. Dryness grades were significantly higher in winter ($p < 0.001$). There was no significant difference in erythema grades (n.s.).

The observation that dry skin grades were significantly lower in summer was expected. The observation that erythema grades were not significantly different between seasons is in agreement with the results reported by Visscher *et al.* who compared dry skin grades on the hands of health-care workers in winter and late spring and found significantly higher dryness grades in the winter with no significant difference in erythema grades between seasons (6).

SC COHESION

In addition to being a convenient way to sample the SC for analysis, tape stripping can be used to assess the cohesiveness of the SC. Each strip removes less protein from SC that is more cohesive. An advantage of the SquameScan™ system is that it accurately measures SC removed nondestructively (25,36,37), and the tapes can still be used for further analysis. Figure 3 shows protein removed by each tape strip in winter and summer. Each tape removed significantly more SC in winter compared to summer ($p < 0.001$ for each strip level). The difference was especially marked for the first five tape strips.

Total protein in strip 4 measured by BCA protein analysis in addition to SquameScan™ is shown in Figure 4.

Significantly, more protein was seen on the winter tape 4 strips and the winter/summer ratio for tape 4 was 1.9 by BCA protein analysis and 2.0 by SquameScan™ (Figure 3).

The finding of reduced SC cohesion in winter dry skin is consistent with the results of Lu *et al.* (38) who reported results comparing "cosmetic dry skin" to normal skin on the legs analyzed by tape stripping with D-Squame discs. More protein was removed by each of the first 10 strips of dry skin with the differences being largest in the first strips. In order for SC to desquamate properly, the desmosomes (corneodesmosomes) that bind individual corneocytes together must break down, and this process has been shown to be impeded in winter dry skin (2,39). It is probable that each tape strip removes more SC, especially from the less compact outer layers of the SC (40,41), which may be more prominent in dry skin because the desmosomes are not completely broken down in the outer layers of dry skin (2,39,42).

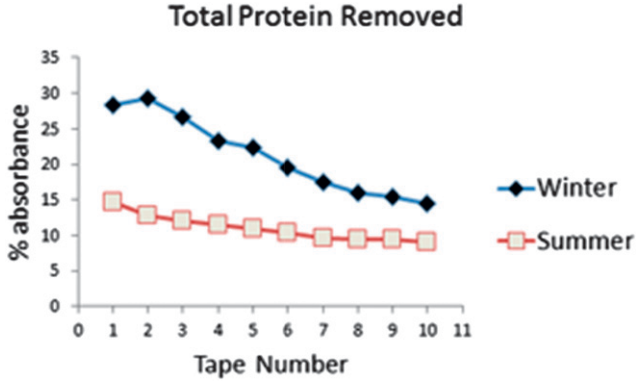

Figure 3. SC cohesiveness was evaluated by measuring protein removed by each of the 10 sequential tape strips nondestructively using the SquameScan™ 850A infrared densitometer. Significantly ($p < 0.0001$) more protein was removed by each strip in winter compared to summer.

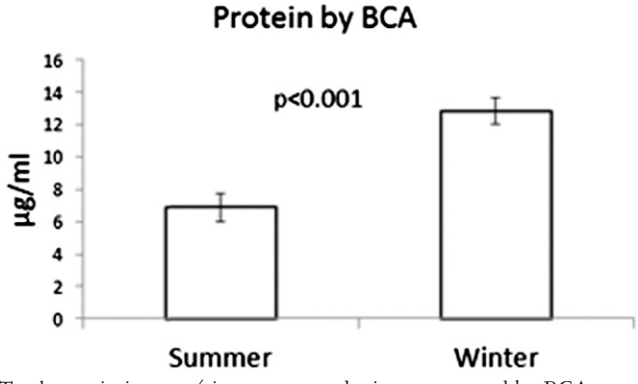

Figure 4. Total protein in tape 4 in summer and winter measured by BCA protein analysis.

The higher sensitivity of skin to irritants in winter may also be at least partly related to SC cohesiveness. Tumor necrosis factor-α (TNF-α) gene polymorphism makes skin more susceptible to surfactant (19,43) and sensory irritation (44). Davis *et al.* (45) reported that D-Squame tape stripping removed significantly more SC from subjects with this polymorphism indicating that they had reduced SC cohesion compared to subject without TNF-α polymorphism.

NMF COMPONENTS

The NMFs of the SC are primarily free amino acids derived from the breakdown of filaggrin (46–49) though lactate may also play a role in keeping the SC hydrated (14). NMF in SC has been investigated by several different workers in various contexts, including body site variations (9,50), effects of age (51), dry skin (51,52), treatments such as soaking and lipid extraction (53,54), and barrier perturbation and repair (55).

One of the most important NMF components is the very hygroscopic compound PCA formed by the nonenzymatic cyclization of free glutamine produced during filaggrin hydrolysis (56). PCA is reduced in the lesioned skin of patients with atopic dermatitis (AD), along with reduced skin hydration (57), and in the skin of the elderly (42). Feng *et al.* reported that PCA levels are reduced in the SC of subjects with "cosmetic dry skin" compared to normal subjects, and PCA levels have been reported to be higher in subjects with lower visual grades for dry skin (52).

Tapes 3 and 10 were analyzed for the NMF components (free amino acids, t-UA, and PCA) that arise primarily from the breakdown of filaggrin (46,48,49). Figure 5A shows PCA levels normalized to protein in tapes 3 and 10 in summer and winter. The difference between strips was not significant but the difference between summer and winter was highly significant at both strips 3 and 10. Other amino acids in strip 10 are shown in Figure 5B. There were small but statistically significant increases in glycine, proline, serine, and the sum of amino acids in summer compared to winter, and t-UA and histidine were lower in summer compared to winter.

The lower level of t-UA in summer is consistent with the level reported by Egawa and Tagami (9), and may be due to cis–trans isomerization of UA under the effect of ultraviolet

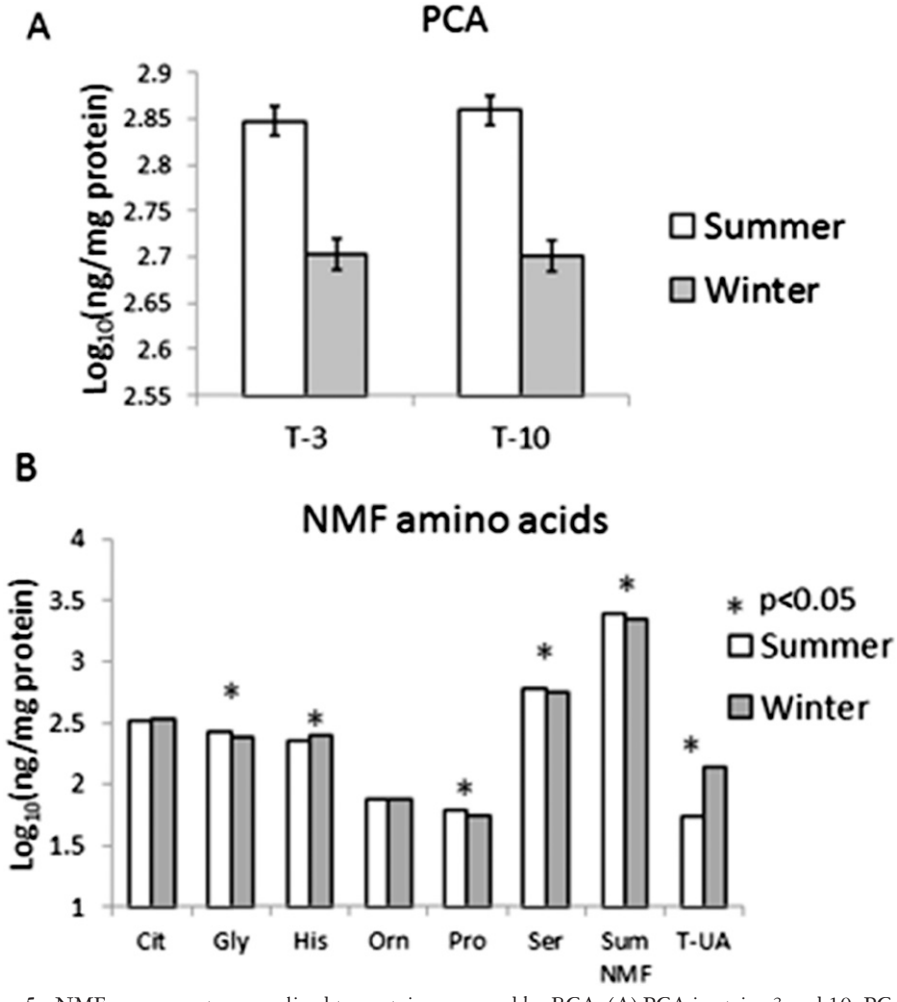

Figure 5. NMF components normalized to protein measured by BCA. (A) PCA in strips 3 and 10, PCA was significantly higher in summer in both strips ($p < 0.001$), and the levels were not significantly different between strips 3 and 10. (B) Amino acids from strip 10. Glycine ($p = 0.006$), proline ($p = 0.037$), serine ($p = 0.017$), and the sum of amino acids analyzed (sum NMF, $p = 0.018$) were significantly higher in summer. Histidine ($p = 0.035$) and t-UA ($p < 0.001$) were significantly higher in winter.

light in summer as reported by de Fine *et al.* (58). In contrast, our results on PCA and free amino acids are not consistent with those of Egawa and Tagami who reported that PCA and free amino acids were not higher in summer compared to other seasons on the forearms and cheeks of Japanese subjects. However, subjects whose skin did not "feel dry" by self-assessment in both spring and autumn were found to have higher levels of NMF than those with skin that did "feel dry" (9). The difference may be due to the different body site tested, the different methodology (confocal Raman vs. analysis of tape strips) or to the fact that we selected subjects with at least moderately dry skin in the winter to participate in the present study.

STRUCTURAL PROTEINS

Figure 6 shows results for the key structural proteins keratin-1,10,11. These keratins are formed during terminal differentiation of keratinocytes to form the SC (59–61). Keratin-1,10,11 levels were significantly higher in summer compared to winter. Involucrin (not shown) was not significantly different between the seasons.

The increased level of structural proteins is consistent with improved barrier function in summer. A previous report that keratin 10 is lower in dry skin compared to normal skin (62) is consistent with our findings of lower keratin-1,10,11 in winter.

CYTOKINES

Inflammatory cytokines are elevated in irritated skin, and the ratio of IL-1ra:IL-1α is especially sensitive to irritation (18,19,22). Figure 7 shows that the ratio of IL-1ra:IL-1α was significantly higher in winter compared to summer.

The reduced IL-1ra:IL-1α ratio is consistent with healthier and less inflamed skin in summer. It is interesting that erythema grades were not lower in summer even though IL-1ra:IL-1α ratio was reduced, indicating some increased level of underlying inflammation in dry skin that does not exhibit as increased redness. This may represent an example of what Kligman called invisible dermatoses (63).

SC LIPIDS

The multilamellar intracellular lipids of the SC are the main barrier to water loss to the environment allowing us to live in a terrestrial environment (64–67). SC lipid profiles have been found to be altered in dry skin (68), surfactant-damaged skin (2,68,69), and in patients with AD (70–74).

Ceramides are long-chain fatty acids amide linked to one of four sphingoid bases. Three types of fatty acids can also be involved in SC lipid profiles, nonhydroxy fatty acids [N], α-hydroxy fatty acids [A], and esterified ω-hydroxy fatty acids [EO]. This leads to the 12

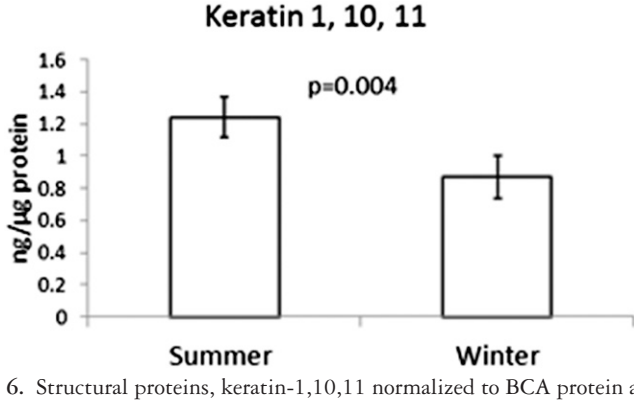

Figure 6. Structural proteins, keratin-1,10,11 normalized to BCA protein analysis.

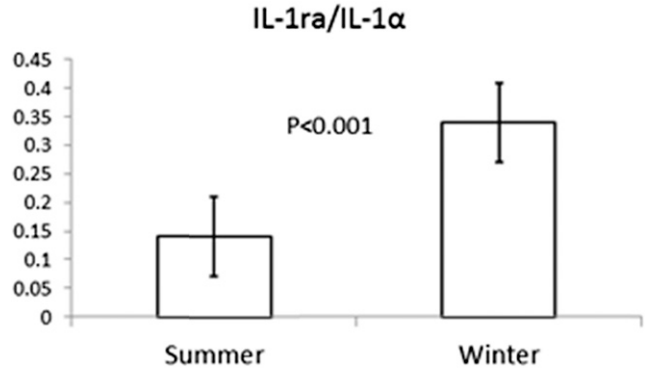

Figure 7. IL-1ra:IL-1α ratio. The ratio was significantly higher in winter.

classes of ceramides as shown in Table II (72,75,76). Ceramides, especially long-chain ceramides such as those in the ceramide EOS class (formerly ceramide 1), have been found to be especially important for the proper formation of the SC barrier lipid structure (77,78).

Results of the lipid analysis sorted by largest difference between summer and winter are given in Table III. The ceramide nomenclature is given in Table II: C30_C18_2 is a ceramide EO with 30 carbon chain esterified to a C18 fatty acid with two double bonds, N30_0_P18

Table II
Fatty Acids and Sphingoid Bases That Can Combine to Produce 12 Possible Ceramide Classes: 11 Are Known in Skin.*

Fatty acid / Sphingoid	Non-hydroxy fatty acid [N]	α-hydroxy fatty acid [A]	Esterified ω-hydroxy fatty acid [EO]
Dihydrosphingosine [DS]	CER[NDS]	CER[ADS]	CER[EODS] (not yet identified in SC)
Sphingosine [S]	CER[NS]	CER[AS]	CER[EOS]
Phytosphingosine [P]	CER[NP]	CER[AP]	CER[EOP]
6-hydroxy sphingosine [H]	CER[NH]	CER[AH]	CER[EOH]

*Modified from Reference (75) with permission of Allured publishing.

Table III
Lipid Results Sorted by Summer:Winter Ratio

Ceramide	Class	Summer	Winter	Ratio (Summer:Winter)	% Increase (Summer versus Winter)
C30_C18_2	Ceramide-EOS	2.08	0.95	2.19	119
N30_0_P18	Ceramide-NP	5.06	2.34	2.17	117
C32_C18_1	Ceramides-EOS	0.23	0.11	2.11	111
C18_2	Fatty acid	2.78	1.39	2.00	100
N28_0_P18	Ceramide-NP	9.11	4.57	1.99	99
C32_C18_2	Ceramide-EOS	1.11	0.56	1.99	99
C16_1	Fatty acids	4.16	2.13	1.95	95
N26_0_P18	Ceramide-NP	6.38	3.31	1.93	93
C24_0	Fatty acids	45.87	24.25	1.89	89
N24_0_P18	Ceramide-NP	6.59	3.56	1.85	85
Cholesterol	Sterol	284.17	156.44	1.82	82
C30_C18_1	Ceramide-EOS	0.31	0.17	1.82	82
C18_1	Fatty acids	9.52	5.27	1.80	80
Ceramides	Ceramides-all	43.37	24.16	1.79	79
P18	Sphingosine bases	1.56	0.88	1.78	78
A26_0_P18	Ceramide-AP	1.28	0.72	1.77	77
A24_0_P18	Ceramide-AP	0.93	0.52	1.77	77
C14_0	Fatty acids	6.07	3.44	1.76	76
C22_0	Fatty acids	12.94	7.45	1.74	74
N24_0_DS18	Ceramide-NDS	1.46	0.87	1.67	67
N26_0_DS18	Ceramide-NDS	2.90	1.85	1.57	57
Sphingoid bases	Sphingosine bases	3.23	2.10	1.54	54
TOTAL FA	Fatty Acids-All	153.89	104.98	1.47	47
A26_0_H18	Ceramide-AH	2.30	1.67	1.38	38
DS18	Sphingosine bases	0.39	0.29	1.36	36
S18	Sphingosine bases	1.19	0.90	1.32	32
C16_0	Fatty acids	34.84	26.56	1.31	31
A24_0_H18	Ceramide-AH	1.23	0.96	1.28	28
A16_0_S18	Ceramide-AS	1.50	1.31	1.14	14
C18_0	Fatty acids	29.62	29.07	1.02	2
Cholesterol-SO_4	Sterol-sulfate	9.63	10.19	0.94	-6

is a saturated C30-NP ceramide with an 18 carbon α-hydroxy fatty acid, C22_0 is an unsaturated C22 fatty acid, C18_1 is a fatty acid with one double bond (oleic acid), etc.

We quantified ceramides, cholesterol, cholesterol sulfate, and fatty acids in winter and summer in tapes 6 and 7 (pooled). Results of lipids log normalized for protein, sorted by ratio and % increase summer/winter sorted from highest % change to lowest are shown in Table III.

Figure 8 is a spider graph showing all of the lipid results as the percentage increase from winter to summer.

All lipids quantified except stearic acid (C18_0) (+2%) and cholesterol sulfate (-6%) increased significantly in summer. Among the ceramides, EOS and NP ceramides increased more than AH and AS ceramides, and the short-chain ceramide A16_0_S18 increased the least.

Our results are in agreement with those of Rogers *et al.* (15) and Conti *et al.* (16) who reported increase in ceramide 1 (EOS), fatty acids, and cholesterol in summer compared to winter.

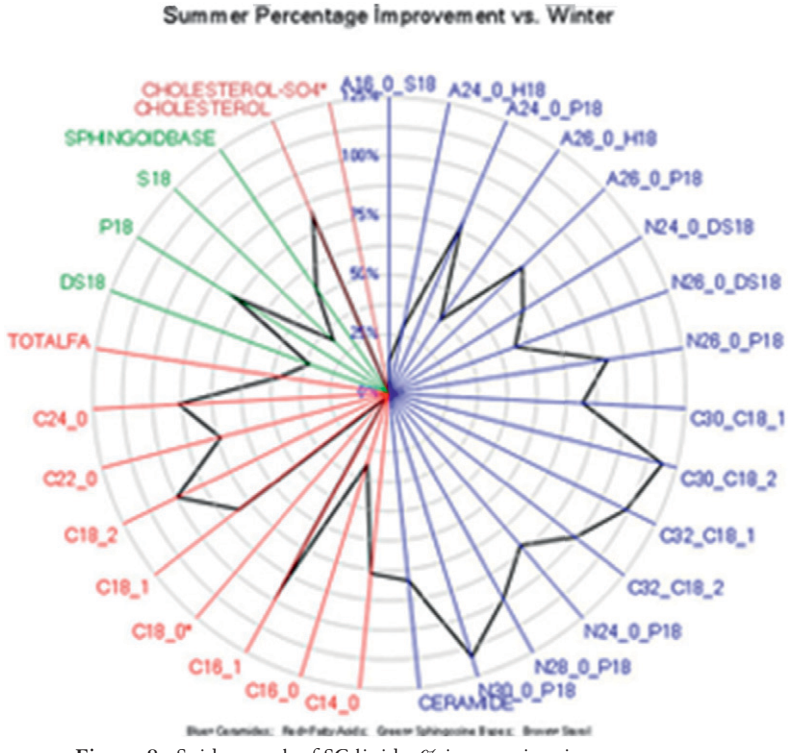

Figure 8. Spider graph of SC lipids, % increase in winter to summer.

Janssens *et al.* (72) reported that TEWL in AD patients is inversely proportional to the level of longer chain ceramides but increases with the presence of shorter chain ceramides, especially the AS ceramides. In this respect, it is interesting to compare the ratios of ceramides from the data given in Table III. Table IV shows the ceramides that Janssens *et al.* found to be correlated with reduced TEWL. Ceramides that were reported to be associated with increased TEWL are shown in Table V.

Table IV
Long Chain Ceramides

Ceramide	Class	Summer	Winter	Summer:Winter
C30_C18_1	Ceramide-EOS	0.31	0.17	1.82
C30_C18_2	Ceramide-EOS	2.08	0.95	2.19
C32_C18_1	Ceramides-EOS	0.23	0.11	2.09
C32_C18_2	Ceramide-EOS	1.11	0.56	1.98
N24_0_DS18	Ceramide-NDS	1.46	0.87	1.68
N24_0_P18	Ceramide-NP	6.59	3.56	1.85
N26_0_DS18	Ceramide-NDS	2.90	1.85	1.57
N26_0_P18	Ceramide-NP	6.38	3.31	1.93
N28_0_P18	Ceramide-NP	9.11	4.57	1.99
N30_0_P18	Ceramide-NP	5.06	2.34	2.16
Total	Long chain	35.23	18.29	**1.93**

Table V
Short-Chain Ceramides

Ceramide	Class	Summer	Winter	Summer:Winter
A16_0_S18	Ceramide-AS	1.50	1.31	1.15
A24_0_H18	Ceramide-AH	1.23	0.96	1.28
A24_0_P18	Ceramide-AP	0.93	0.52	1.79
A26_0_H18	Ceramide-AH	2.30	1.67	1.38
A26_0_P18	Ceramide-AP	1.28	0.72	1.78
Total	Short chain	7.24	5.18	**1.40**

Ceramides that were reported to decrease TEWL showed a summer:winter ratio of 1.9 while the summer:winter ratio of short-chain ceramides that were reported to decrease TEWL was 1.4. The long-chain:short-chain ratio of the ceramides studied was 4.9 in summer and 3.5 in winter. The increase in the ratio of ceramides reported to correlate with reduced TEWL to those reported to decrease TEWL is consistent with the significantly reduced TEWL in summer reported in Figure 1B.

Cholesterol increased in summer while cholesterol sulfate declined slightly. The cholesterol:cholesterol sulfate ratio nearly doubled between winter and summer. This may also play a role in the improved barrier function and skin condition in summer. Cholesterol is an important component of the SC barrier while cholesterol sulfate can disrupt the barrier (79) and may contribute to skin scaling by inhibiting desmosome digestion (80).

CONCLUSIONS

Our results indicate that many factors may contribute to generally improved skin health in summer compared to winter. Some of the seasonal differences in this report may be more marked than some of those in other reports because we did select subjects with dry skin in the winter and then followed those same subjects in the summer. Thus, we picked subjects with some tendency to develop winter dry skin. However, these are the subjects most in need of cleansing and moisturizing products formulated to have optimal beneficial effects on the skin, and this is a goal of much research and development in the cosmetic industry.

The significantly better barrier function of the skin in summer (lower TEWL) may be the result of improved levels and ratios of SC lipids. The higher level of keratin-1,10,11 may also contribute to a better summer barrier. Engelke *et al*. did not find a correlation between TEWL and dry skin in their study even though K10 was lower in dry skin. Perhaps this is because all of their measurements were carried out in winter (62).

Improved skin hydration results at least in part from higher levels of PCA and NMF free amino acids and perhaps from higher water content due to the improved barrier to water loss. Lactate, which was not measured, has also been found to increase in the summer (9,14) and may contribute to higher SC hydration in summer as well.

The lower IL-1ra:IL-1α ratio, an indicator of skin inflammation, in the summer indicates that winter dry skin may be slightly inflamed.

ACKNOWLEDGMENTS

We are grateful for many helpful discussions with Ed Smith, Kathy Kerr, and Robert Binder from Procter & Gamble.

REFERENCES

(1) K. D. Ertel, B. H. Keswick, and P. B. Bryant, A forearm controlled application technique for estimating the relative mildness of personal cleansing products, *J. Soc. Cosmet. Chem.,* **46,** 67–76 (1995).
(2) A. V. Rawlings, A. Watkinson, J. Rogers, A. Mayo, J. Hope, and I. A. Scott, Abnormalities in stratum corneum structure, lipid composition, and desmosome degradation in soap-induced winter xerosis, *J. Soc. Cosmet. Chem.,* **45,** 203–220 (1994).
(3) D. A. Basketter, H. A. Griffiths, X. M. Wang, K. P. Wilhelm, and J. McFadden, Individual, ethnic and seasonal variability in irritant susceptibility of skin: The implications for a predictive human patch test, *Contact Dermatitis,* **35,** 208–213 (1996).
(4) J. Canning, B. Barford, D. Sullivan, R. Wickett, and M. Visscher, Use of digital photography and image analysis techniques to quantify erythema in health care workers, *Skin Res. Technol.,* **15,** 24–34 (2009).
(5) E. Larson, K. J. McGinley, G. L. Grove, J. J. Leyden, and G. H. Talbot, Physiologic, microbiologic, and seasonal effects of handwashing on the skin of health care personnel, *Am. J. Infect. Control,* **14,** 51–59 (1986).
(6) M. Visscher, J. Canning, D. Said, R. Wickett, and P. Bondurant, Effect of hand hygiene regimens on skin condition in health care workers, *Am. J. Infect. Control,* **34,** S111–S123 (2006).
(7) M. J. Wan, X. Y. Su, Y. Zheng, Z. J. Gong, J. L. Yi, Y. Zhao, X. M. Guan, and W. Lai, Seasonal variability in the biophysical properties of forehead skin in women in Guangzhou City, China, *Int. J. Dermatol.,* **54,** 12–29 (2014).
(8) D. Black, A. Del Pozo, J. M. Lagarde, and Y. Gall, Seasonal variability in the biophysical properties of stratum corneum from different anatomical sites, *Skin Res. Technol.,* **6,** 70–76 (2000).
(9) M. Egawa and H. Tagami, Comparison of the depth profiles of water and water-binding substances in the stratum corneum determined in vivo by Raman spectroscopy between the cheek and volar forearm skin: Effects of age, seasonal changes and artificial forced hydration, *Br. J. Dermatol.,* **158,** 251–260 (2008).
(10) K. Kikuchi, H. Kobayashi, I. Le Fur, E. Tschachler, and H. Tagami, The winter season affects more severely the facial skin than the forearm skin: Comparative biophysical studies conducted in the same Japanese females in later summer and winter, *Exogenous Dermatol.,* **1,** 32–38 (2002).
(11) N. Muizzuddin, M. Ingrassia, K. D. Marenus, D. H. Maes, and T. Mammone, Effect of seasonal and geographical differences on skin and effect of treatment with an osmoprotectant: Sorbitol, *J. Cosmet Sci.,* **64,** 165–174 (2013).
(12) H. Qiu, X. Long, J. C. Ye, J. Hou, J. Senee, A. Laurent, R. Bazin, F. Flament, A. Adam, J. Coutet, and B. Piot, Influence of season on some skin properties: Winter vs. summer, as experienced by 354 Shanghaiese women of various ages, *Int. J. Cosmet. Sci.,* **33,** 377–383 (2011).
(13) G. W. Nam, J. H. Baek, J. S. Koh, and J. K. Hwang, The seasonal variation in skin hydration, sebum, scaliness, brightness and elasticity in Korean females, *Skin Res. Technol.,* **21,** 1–8 (2015).
(14) N. Nakagawa, S. Sakai, M. Matsumoto, K. Yamada, M. Nagano, T. Yuki, Y. Sumida, and H. Uchiwa, Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects, *J. Invest. Dermatol.,* **122,** 755–763 (2004).
(15) J. Rogers, C. Harding, A. Mayo, J. Banks, and A. Rawlings, Stratum corneum lipids: The effect of ageing and the seasons, *Arch. Dermatol. Res.,* **288,** 765–770 (1996).
(16) A. Conti, J. Rogers, P. Verdejo, C. R. Harding, and A. V. Rawlings, Seasonal influences on stratum corneum ceramide 1 fatty acids and the influence of topical essential fatty acids, *Int. J. Cosmet. Sci.,* **18,** 1–12 (1996).
(17) J. Ishikawa, Y. Shimotoyodome, S. Ito, Y. Miyauchi, T. Fujimura, T. Kitahara, and T. Hase, Variations in the ceramide profile in different seasons and regions of the body contribute to stratum corneum functions, *Arch. Dermatol. Res.,* **305,** 151–162 (2013).
(18) C. M. de Jongh, M. M. Verberk, S. W. Spiekstra, S. Gibbs, and S. Kezic, Cytokines at different stratum corneum levels in normal and sodium lauryl sulphate-irritated skin, *Skin Res. Technol.,* **13,** 390–398 (2007).

(19) C. M. de Jongh, M. M. Verberk, C. E. Withagen, J. J. Jacobs, T. Rustemeyer, and S. Kezic, Stratum corneum cytokines and skin irritation response to sodium lauryl sulfate, *Contact Dermatitis,* **54,** 325–333 (2006).
(20) K. Kerr, T. Darcy, J. Henry, H. Mizoguchi, J. R. Schwartz, S. Morrall, T. Filloon, R. Wimalasena, G. Fadayel, and K. J. Mills, Epidermal changes associated with symptomatic resolution of dandruff: Biomarkers of scalp health, *Int .J. Dermatol.,* **50,** 102–113 (2011).
(21) V. Narendran, M. O. Visscher, I. Abril, S. W. Hendrix, and S. B. Hoath, Biomarkers of epidermal innate immunity in premature and full-term infants, *Pediatr. Res.,* **67,** 382–386 (2010).
(22) M. O. Visscher, D. Said, and R. Wickett, Stratum corneum cytokines, structural proteins, and transepidermal water loss: Effect of hand hygiene, *Skin Res. Technol.,* **16,** 229–236 (2010).
(23) T. B. Fitzpatrick, The validity and practicality of sun-reactive skin types I through VI, *Arch. Dermato.,* **124,** 869–871 (1988).
(24) M. F. Lukacovic, F. E. Dunlap, S. E. Michaels, M. O. Visscher, and D. D. Watson, Forearm wash test to evaluate the clinical mildness of cleansing products, *J. Soc. Cosmet. Chem.,* **39,** 355–366 (1988).
(25) R. Voegeli, J. Heiland, S. Doppler, A. V. Rawlings, and T. Schreier, Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry, *Skin Res. Technol.,* **13,** 242–251 (2007).
(26) E. Berardesca, EEMCO guidance for the assessment of stratum corneum hydration: Electrical methods, *Skin Res. Technol.,* **3,** 126–132 (1997).
(27) J. W. Fluhr, M. Gloor, S. Lazzerini, P. Kleesz, R Grieshaber, and E. Berardesca, Comparative study of five instruments measuring stratum corneum hydration (Corneometer CM 820 and CM 825, Skicon 200, Nova DPM 9003, DermLab). Part I. In vitro, *Skin Res. Technol.,* **5,** 161–170 (1999).
(28) J. W. Fluhr, M. Gloor, S. Lazzerini, P. Kleesz, R Grieshaber, and E. Berardesca, Comparative study of five instruments measuring stratum corneum hydration (Corneometer CM 820 and CM 825, Skicon 200, Nova DPM 9003, DermLab). Part II. In vivo, *Skin Res. Technol.,* **5,** 171–178 (1999).
(29) P. Clarys, A. O. Barel, and B. Gabard, Non-invasive electrical measurements for the evaluation of the hydration state of the skin: Comparison between three conventional instruments—The Corneometer, the Skicon and the Nova DPM, *Skin Res. Technol.,* **5,** 14–20 (1999).
(30) B. M. Morrison and D. Scala, Comparison of instrumental measurements of skin hydration, *J. Toxicol. Cut. Ocular. Toxicol.,* **15,** 305–314 (1996).
(31) J. W. Fluhr, K. R. Feingold, and P. M. Elias, Transepidermal water loss reflects permeability barrier status: Validation in human and rodent in vivo and ex vivo models, *Exp. Dermatol.*, **15,** 483–492 (2006).
(32) K. Hammarlund, G. E. Nilsson, P. A. Oberg, and G. Sedin, Transepidermal water loss in newborn infants. I. Relation to ambient humidity and site of measurement and estimation of total transepidermal water loss, *Acta. Paediatr. Scand.,* **66,** 553–562 (1977).
(33) J. L. Leveque, J. C. Garson, and J. de Rigal, Transepidermal water loss from dry and normal skim, *J. Soc. Cosmet. Chem.,* **30,** 333–373 (1979).
(34) J. Pinnagoda, R. A. Tupker, T. Agner, and J. Serup, Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis, *Contact Dermatitis,* **22,** 164–178 (1990).
(35) J. Pinnagoda, R. A. Tupker, P. J. Coenraads, and J. P. Nater, Prediction of susceptibility to an irritant response by transepidermal water loss, *Contact Dermatitis,* **20,** 341–346 (1989).
(36) T. Hahn, S. Hansen, D. Neumann, K. H. Kostka, C. M. Lehr, L. Muys, and U. F. Schaefer, Infrared densitometry: A fast and non-destructive method for exact stratum corneum depth calculation for in vitro tape-stripping, *Skin Pharmacol. Physiol.,* **23,** 183–192 (2010).
(37) D. Mohammed, Q. Yang, R. H. Guy, P. J. Matts, J. Hadgraft, and M. E. Lane, Comparison of gravimetric and spectroscopic approaches to quantify stratum corneum removed by tape-stripping, *Eur. J. Pharm. Biopharm.,* **82,** 171–174 (2012).
(38) N. Lu, P. Chandar, D. Tempesta, C. Vincent, J. Bajor, and H. McGuiness, Characteristic differences in barrier and hygroscopic properties between normal and cosmetic dry skin. I. Enhanced barrier analysis with sequential tape-stripping, *Int. J. Cosmet. Sci.,* **36,** 167–174 (2014).
(39) M. Simon, D. Bernard, A. M. Minondo, C. Camus, F. Fiat, P. Corcuff, R. Schmidt, and G. Serre, Persistence of both peripheral and non-peripheral corneodesmosomes in the upper stratum corneum of winter xerosis skin versus only peripheral in normal skin, *J. Invest. Dermatol.,* **116,** 23–30 (2001).
(40) S. J. Chapman and A. Walsh, Desmosomes, corneosomes and desquamation. An ultrastructural study of adult pig epidermis, *Arch. Dermatol. Res.,* **282,** 304–310 (1990).

(41) M. Fartasch, I. D. Bassukas, and T. L. Diepgen, Structural relationship between epidermal lipid lamellae, lamellar bodies and desmosomes in human epidermis: An ultrastructural study, *Br. J. Dermatol.,* **128,** 1–9 (1993).
(42) C. R. Harding, A. Watkinson, A. V. Rawlings, and I. R. Scott, Dry skin, moisturization and corneodesmolysis, *Int. J. Cosmet. Sci.,* **22,** 21–52 (2000).
(43) C. M. de Jongh, S. M. John, D. P. Bruynzeel, F. Calkoen, F. J. van Dijk, L. Khrenova, T. Rustemeyer, M. M. Verberk, and S. Kezic, Cytokine gene polymorphisms and susceptibility to chronic irritant contact dermatitis, *Contact Dermatitis,* **58,** 269–277 (2008).
(44) J. A. Davis, M. O. Visscher, R. R. Wickett, and S. B. Hoath, Role of TNF-a polymorphism-308 in neurosensory irritation, *Int. J. Cosmet. Sci.,* **33,** 105–112 (2011).
(45) J. A. Davis, M. O. Visscher, R. R. Wickett, and S. B. Hoath, Influence of tumour necrosis factor-a polymorphism-308 and atopy on irritant contact dermatitis in healthcare workers, *Contact Dermatitis,* **63,** 320–332 (2010).
(46) C. R. Harding, S. Aho, and C. A. Bosko, Filaggrin—Revisited, *Int. J. Cosmet. Sci.,* **35,** 412–423 (2013).
(47) I. Horii, K. Kawasaki, J. Koyama, Y. Nakayama, K. Nakajima, K. Okazaki, and M. Seiji, Histidine-rich protein as a possible origin of free amino acids of stratum corneum, *Curr. Probl. Dermatol.,* **11,** 301–315 (1983).
(48) A. V. Rawlings, I. R. Scott, C. R. Harding, and P. A. Bowser, Stratum corneum moisturization at the molecular level, *J. Invest. Dermatol.,* **103,** 731–741 (1994).
(49) I. R. Scott, C. R. Harding, and J. G. Barrett, Histidine-rich protein of the keratohyalin granules. Source of the free amino acids, urocanic acid and pyrrolidone carboxylic acid in the stratum corneum, *Biochim. Biophys. Acta.,* **719,** 110–117 (1982).
(50) M. Visscher, M. Robinson, and R. R. Wickett, Regional variation in the free amino acids in the stratum corneum, *J. Cosmet. Sci.,* **61,** 303–309 (2010).
(51) T. M. Jacobson, K. U. Yuksel, J. C. Geesin, J. S. Gordon, A. T. Lane, and R. W. Gracy, Effects of aging and xerosis on the amino acid composition of human skin, *J. Invest. Dermatol.,* **95,** 296–300 (1990).
(52) L. Feng, P. Chandar, N. Lu, C. Vincent, J. Bajor, and H. McGuiness, Characteristic differences in barrier and hygroscopic properties between normal and cosmetic dry skin. II. Depth profile of natural moisturizing factor and cohesivity, *Int. J. Cosmet. Sci.,* **36,** 231–238 (2014).
(53) M. Robinson, M. Visscher, A. Laruffa, and R. Wickett, Natural moisturizing factors (NMF) in the stratum corneum (SC). I. Effects of lipid extraction and soaking, *J. Cosmet. Sci.,* **61,** 13–22 (2010).
(54) M. Robinson, M. Visscher, A. Laruffa, and R. Wickett, Natural moisturizing factors (NMF) in the stratum corneum (SC). II. Regeneration of NMF over time after soaking, *J. Cosmet. Sci.,* **61,** 23–29 (2010).
(55) M. Visscher, M. Robinson, and R. Wickett, Stratum corneum free amino acids following barrier perturbation and repair, *Int. J. Cosmet. Sci.,* **33,** 80–89 (2011).
(56) J. G. Barrett and I. R. Scott, Pyrrolidone carboxylic acid synthesis in guinea pig epidermis, *J. Invest. Dermatol.,* **81,** 122–124 (1983).
(57) M. Jung, J. Choi, S. A. Lee, H. Kim, J. Hwang, and E. H. Choi, Pyrrolidone carboxylic acid levels or caspase-14 expression in the corneocytes of lesional skin correlates with clinical severity, skin barrier function and lesional inflammation in atopic dermatitis, *J. Dermatol. Sci.,* **76,** 231–239 (2014).
(58) Olivarius F. de Fine, H. C. Wulf, J. Crosby, and M. Norval, Seasonal variation in urocanic acid isomers in human skin, *Photochem. Photobiol.,* **66,** 119–123 (1997).
(59) D. R. Roop, P. Hawley-Nelson, C. K. Cheng, and S. H. Yuspa, Keratin gene expression in mouse epidermis and cultured epidermal cells, *Proc. Natl. Acad. Sci. U.S.A.,* **80,** 716–720 (1983).
(60) J. Schweizer, M. Kinjo, G. Furstenberger, and H. Winter, Sequential expression of mRNA-encoded keratin sets in neonatal mouse epidermis: Basal cells with properties of terminally differentiating cells, *Cell,* **37,** 159–170 (1984).
(61) M. Regnier, P. Vaigot, M. Darmon, and M. Prunieras, Onset of epidermal differentiation in rapidly proliferating basal keratinocytes, *J. Invest. Dermatol.,* **87,** 472–476 (1986).
(62) M. Engelke, J. M. Jensen, S. Ekanayake-Mudiyanselage, and E. Proksch, Effects of xerosis and ageing on epidermal proliferation and differentiation, *Br. J. Dermatol.,* **137,** 219–225 (1997).
(63) A. M. Kligman, The invisible dermatoses, *Arch. Dermatol.,* **127,** 1375–1382 (1991).
(64) D. T. Downing, M. E. Stewart, P. W. Wertz, S. W. Colton, W. Abraham, and J. S. Strauss, Skin lipids: An update, *J. Invest. Dermatol.,* **88 (Suppl),** 2s–6s (1987).
(65) P. M. Elias, Epidermal lipids, barrier function, and desquamation, *J. Invest. Dermatol.,* **80 (Suppl),** 44s-49s (1983)
(66) K. C. Madison, Barrier function of the skin: "la raison d'etre" of the epidermis, *J. Invest. Dermatol.,* **121,** 231–241 (2003).

(67) P. W. Wertz, Lipids and barrier function of the skin, *Acta. Derm. Venereol. Suppl. (Stockh),* **208,** 7–11 (2000).
(68) K. Akimoto, N. Yoshikawa, Y. Higaki, M. Kawashima, and G. Imokawa, Quantitative analysis of stratum corneum lipids in xerosis and asteatotic eczema, *J. Dermatol.,* **20,** 1–6 (1993).
(69) A. W. Fulmer and G. J. Kramer, Stratum corneum lipid abnormalities in surfactant-induced dry scaly skin, *J. Invest. Dermatol.,* **86,** 598–602 (1986).
(70) A. di Nardo, P. Wertz, A. Giannetti, and S. Seidenari, Ceramide and cholesterol composition of the skin of patients with atopic dermatitis, *Acta. Derm. Venereol.,* **78,** 27–30 (1998).
(71) G. Imokawa, Lipid abnormalities in atopic dermatitis, *J. Am. Acad. Dermatol.,* **45,** S29–S32 (2001).
(72) M. Janssens, J. van Smeden, G. S. Gooris, W. Bras, G. Portale, P. J. Caspers, R. J. Vreeken, T. Hankemeier, S. Kezic, R. Wolterbeek, A. P. Lavrijsen, and J. A. Bouwstra, Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients, *J. Lipid Res.,* **53,** 2755–2766 (2012).
(73) M. Janssens, J. van Smeden, G. S. Gooris, W. Bras, G. Portale, P. J. Caspers, R. J. Vreeken, S. Kezic, A. P. Lavrijsen, and J. A. Bouwstra, Lamellar lipid organization and ceramide composition in the stratum corneum of patients with atopic eczema, *J. Invest. Dermatol.,* **131,** 2136–2138 (2011).
(74) J. van Smeden, M. Janssens, E. C. Kaye, P. J. Caspers, A. P. Lavrijsen, R. J. Vreeken, and J. A. Bouwstra, The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients, *Exp. Dermatol.,* **23,** 45–52 (2014).
(75) M. Farwick, P Lersch, B. Santonnat, K. Korevaar, and A. V. Rawlings, Developments in ceramide identification, synthesis, function and nomenclature, *Cosmetics Toiletries,* **124,** 63–72 (2009).
(76) J. Ishikawa, H. Yoshida, S. Ito, A. Naoe, T. Fujimura, T. Kitahara, Y. Takema, C. Zerweck, and G. L. Grove, Dry skin in the winter is related to the ceramide profile in the stratum corneum and can be improved by treatment with a Eucalyptus extract, *J. Cosmet. Dermatol.,* **12,** 3–11 (2013).
(77) J. A. Bouwstra, G. S. Gooris, F. E. Dubbelaar, A. M. Weerheim, A. P. Ijzerman, and M. Ponec, Role of ceramide 1 in the molecular organization of the stratum corneum lipids, *J. Lipid Res.,* **39,** 186–196 (1998).
(78) E. H. Mojumdar, Z. Kariman, L. van Kerckhove, G. S. Gooris, and J. A. Bouwstra, The role of ceramide chain length distribution on the barrier properties of the skin lipid membranes, *Biochim. Biophys. Acta.,* **1838,** 2473–2483 (2014).
(79) E. Zettersten, M. Q. Man, J. Sato, M. Denda, A. Farrell, R. Ghadially, M. L. Williams, K. R. Feingold, and P. M. Elias, Recessive x-linked ichthyosis: role of cholesterol-sulfate accumulation in the barrier abnormality, *J. Invest. Dermatol.,* **111,** 784–790 (1998).
(80) J. Sato, M. Denda, J. Nakanishi, J. Nomura, and J. Koyama, Cholesterol sulfate inhibits proteases that are involved in desquamation of stratum corneum, *J. Invest. Dermatol.,* **111,** 189–193 (1998).